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A mismatch between signal transmission efficacy and mating success calls into question the function of complex signals

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Variation in transmission characteristics of signalling environments is hypothesized to influence the evolution of signalling behaviour, signal form and sensory systems of animals. However, many animals communicate across multiple signalling environments, raising the possibility that some displays have evolved explicitly to enable communication across heterogeneous signalling environments. In the present paper, we explored multiple potential impacts of the signalling environment on courtship displays in the wolf spider *Schizocosa retrorsa*. Males of this species court females on a range of substrate types using a combination of vibratory and visual signals. Through a series of experiments, we investigated (1) activity patterns and male microhabitat use, (2) component-specific vibratory signal transmission across natural substrate types and (3) copulation success across substrate types and light levels. We found that, in the laboratory, (1) female and male *S. retrorsa* are most active during daylight hours, and mature males resided and courted most on leaf litter, as compared to their natural habitat types of pine litter or sand; (2) male vibratory courtship signals transmitted best on leaf litter, yet (3) males obtained the highest copulation success on sand, regardless of light level. Our results demonstrate that copulation in *S. retrorsa* is more likely to occur in environments with suboptimal vibratory signal transmission, irrespective of visual signal transmission. We suggest that these results point to (1) a minor role of bimodal (vibratory and visual) courtship signalling in *S. retrorsa*, (2) the importance of an additional signalling modality (most likely air particle movement), (3) a role of other substrate-dependent factors (e.g. predation risk), and/or (4) a reversed female preference for vibratory signalling.

Effective communication between animals is dependent upon the successful transmission and reception of signals, which are in turn influenced by the physical properties of the signalling environment. Indeed, optimizing signal form to best match a given signalling environment has been considered one of the major forces driving signal evolution (Boughman, 2002; Endler, 1992; Gerhardt, 1994). Due to variation in transmission characteristics among microhabitat patches within an animal's natural habitat range, selection has been hypothesized to lead to signals that are well matched to the physical properties of specific signalling environments or locations (Boughman, 2002; Cummings, 2007; Elias, 2006; Elias, Land, Mason, & Hoy, 2006; Elias, Mason, & Hebets, 2010; Elias, Mason, & Hoy, 2004; Endler, 1992; Ng, Landeen, Logsdon, & Glor, 2013). Additionally, habitat-specific selection is hypothesized to influence the tuning of receivers' sensory systems, which can ultimately lead to a tight alignment with the best-transmitted range of conspecific signals (i.e. sensory drive hypothesis) (Boughman, 2002; Endler, 1992; Seehausen et al., 2008; Witte, Farris, Ryan, & Wilczynski, 2005). Under sensory drive then, variation in transmission characteristics among habitats is expected to exert selection on signal evolution and signalling habitat specialization to minimize signal degradation and maximize signal perception by receivers (Boughman, 2001, 2002; Endler, 1992). Tests of this hypothesis, however, require consideration of the effects of habitat-specific variation in signalling behaviour and/or receiver responses on communication.

Many animals communicate in heterogeneous signalling environments consisting of multiple microhabitats, changing light environments and/or different microclimates. Even within a specific

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habitat, transmission characteristics can vary greatly across spatial and temporal scales, particularly for small animals. For example, the physical properties of both signallers (e.g. size) and signals (e.g. physical form or signalling modality; e.g. vibratory, chemical) can influence amplitude, attenuation and overall signalling structure (e.g. chemical composition, frequency spectra) (Endler, 1993). Furthermore, transmission properties may vary over time of day – e.g. most visual signals will transmit further under daylight than moonlight conditions. Thus, the effective transmission and reception of communication displays will vary with the specific location and timing of signal production within a given habitat.

Animals can overcome the challenges associated with environmental variability by (1) adjusting signalling behaviour, such as the location and/or timing of signalling and/or (2) incorporating signals or components with more than one distinct physical form – multimodal signalling (Bro-Jørgensen, 2010; Candolin, 2003; Elias & Mason, 2014; Hebets & McGinley, 2019; Hebets & Papaj, 2005). The expectation of adjusting signalling behaviour has only been explored in a few studies (e.g. Kotiaho, Alatalo, Mappes, & Parri, 2000; McNett, Luan, & Cocroft, 2010; Schmidt & Balakrishnan, 2015; Sueur & Aubin, 2003) while multimodal signalling to overcome environmental heterogeneity has received more attention (reviewed by Bro-Jørgensen, 2010; Hebets & McGinley, 2019). Notably, behavioural plasticity in signalling and multimodal signalling are not mutually exclusive and both may be important in increasing signal efficacy for animals living in highly heterogeneous habitats. Gordon and Uetz (2011), for example, showed that male *Schizocosa ocreata* wolf spiders can actively choose to court on leaf litter and also adjust their multimodal courtship display by increasing visual signals on substrates where the vibratory signal is not transmitted effectively.

Furthermore, a receiver's preference may not always be positively associated with more efficient signal transmission. For instance, female guppies, *Poecilia reticulata*, reverse their preference for conspicuous male coloration in the presence of predators (Evans, Kelley, Ramnarine, & Pilastro, 2002; Gong & Gibson, 1996). In this case, females may avoid conspicuous signals due to predation risk during copulation. Such variation in female preference could evolve due to various ecological factors including predation risk (Evans et al., 2002; Johnson & Basolo, 2003), the risk of heterospecific mating (Sætre et al., 1997) or enhanced paternal care (Griffith, Owens, & Burke, 1999). It could also produce a mating pattern inconsistent with the predictions of sensory drive – e.g. a mismatch between transmission characteristics of the signalling environment and successful communication (i.e. copulation success).

Schizocosa wolf spiders have become a model system for studying the evolution of complex communication (Hebets, Vink, Sullivan-Beckers, & Rosenthal, 2013; Stratton, 2005). Numerous studies have demonstrated a close link between characteristics of signal form and transmission characteristics of the signalling environment. For example, *Schizocosa stridulans* and *Schizocosa floridana* both produce a vibratory signal that transmits well on their natural substrates of leaf litter, and mating trials indicate high mating success on their natural leaf litter habitat – i.e. both species demonstrate a tight signal–substrate match (Elias et al., 2010; Rosenthal, Hebets, Kessler, McGinley, & Elias, 2019). Furthermore, characteristics of the microhabitat influence locomotion in *S. ocreata* (Cady, 1984), and mating success in this latter species can be influenced by visual background complexity and spectral reflectance (Clark, Roberts, Rector, & Uetz, 2011; Uetz, Clark, & Roberts, 2011). Male *S. ocreata* are even known to alter their visual signalling behaviour based on the light environment (Taylor, Roberts, & Uetz, 2005).

Unlike the previously mentioned *Schizocosa* species, prior work on *Schizocosa retrorsa* failed to find a match between substrate-dependent vibratory signal transmission efficacy and mating success (Hebets, Elias, Mason, Miller, & Stratton, 2008; Hebets et al., 2013; Rundus, Santer, & Hebets, 2010). This study thus explores additional aspects of microhabitat use and modality-specific signalling success in an effort to understand the function of bimodal (vibratory and visual) signalling in *S. retrorsa*. Specifically, we explore female and male activity patterns, male substrate use, substrate-specific vibratory signal transmission properties and substrate-specific mating success.

STUDY SYSTEM

The wolf spider *S. retrorsa* can be found across a range of distinct microhabitats. Subadult and mature *S. retrorsa* females and males have been collected on substrates consisting of pine litter, pine litter/sand mix, bare red clay and sand (Hebets, Stratton, & Miller, 1996; E. Hebets, personal observations). Thus, under natural conditions, *S. retrorsa* males presumably court females on a range of substrate types, and they do this using a combination of vibratory and visual signals (Hebets et al., 1996). The vibratory display of *S. retrorsa* consists of two components – pedipalpal drumming and foreleg taps (Hebets et al., 1996). The visual signal components include pigmentation on the femur of mature male forelegs and two dynamic visual displays – foreleg waving and 'push-ups' (Hebets et al., 1996; Stratton, 2005). The visual and vibratory components are structurally linked. Push-ups consist of pedipalpal drumming superimposed with the raising and lowering of the body. Foreleg waving consists of foreleg movements where the forelegs are rapidly raised and lowered, with the forelegs and pedipalps contacting the substrate to produce a percussive impulse (Hebets et al., 1996).

Prior research on *S. retrorsa* found no differences in the importance of vibratory and visual signalling in mating across artificial environments that varied in their vibratory and visual signal transmission properties (Hebets et al., 2013; Rundus et al., 2010). Interestingly, in both studies neither signalling modality appeared necessary for mating success (Hebets et al., 2013; Rundus et al., 2010). Furthermore, the only known predictor of mating success in *S. retrorsa* is leg-waving rate – i.e. males that engaged in more leg waving had an increased likelihood of achieving a mating (Rundus et al., 2010).

Prior work tested the hypothesis that *S. retrorsa*'s multimodal courtship facilitates successful communication across microhabitat types (in terms of a male's ability to secure a mating) by quantifying microhabitat use, vibratory signal transmission and copulation success of males across three substrate types: leaf litter, pine litter and red clay (Hebets et al., 2008). Pine litter and red clay represent habitats upon which the species has been found. Although *S. retrorsa* is not commonly found on leaf litter in the field, typical collection sites are in close proximity/adjacent to available leaf litter habitats (E. Hebets, personal observation). Hebets et al. found that regardless of developmental stage or sex, *S. retrorsa* individuals tended to choose leaf litter first and to reside on leaf litter more than on pine litter or red clay. Adults also moved more frequently among substrate types than did subadults (Hebets et al., 2008). In terms of vibratory signal transmission, differential filtering was observed across the three substrate types. Red clay and pine litter showed a higher-frequency bandwidth at distances further from the sound source (Hebets et al., 2008). Overall, however, vibratory signals sent through leaf litter and pine litter experienced less attenuation than did red clay. Finally, despite the decreased vibratory signal attenuation on leaf litter, copulation success was higher on their natural substrates of pine litter and red clay than it

was on leaf litter. The disconnect between vibratory signal transmission efficacy (best transmission on leaf litter > pine litter > red clay) and copulation success (highest on red clay = pine litter > leaf litter) was proposed to be due to either (1) female preference for high-frequency vibratory courtship signals or (2) differences in efficacy of visual signal transmission. The latter hypothesis proposes that red clay and pine litter have fewer obstacles for visual signalling, thereby increasing the likelihood of females perceiving visual signalling (Hebets et al., 2008). This study directly tests this hypothesis.

As a follow-up to prior work, this series of studies further explores (1) male activity patterns and microhabitat use, (2) component-specific details of vibratory signal transmission across substrate types and (3) male copulation success across light levels, which induce variation in visual signal transmission.

METHODS

Study Animals

For all of the experiments, penultimate-stage females and males were collected from Marshall, Co., Mississippi, U.S.A. The collection site was near Wall Doxey State Park on pipeline E of Old Highway 7 (34°40′N, 89°28′W). The collection date was 28 April 2017 for experiments on activity pattern, vibratory signal transmission and microhabitat choice, and 19 May 2008 for the substrate-dependent mating experiments. Spiders were transported to the laboratory and individually placed in plastic cages (60 × 60 × 80 mm) that had screening on at least two sides to provide a climbing surface, in a controlled light environment (12:12 h light:dark cycle) and constant temperature (25 °C). All cages were covered by opaque tape, so spiders were visually isolated from one another. Water was provided via a cotton wick dipped into a reservoir of water below the cage. Spiders were fed two or three small crickets (*Gryllodes sigillatus*), approximately half the body length of the spider, twice a week and fed one cricket additionally the night before an experiment. Following experiments, we euthanized males by freezing before preserving them in 70% ethanol. All spiders were at the end of their natural life as they only live 1 year, and all bodies were retained as voucher specimens in our collection at the University of Nebraska-Lincoln.

Experiment 1: Activity Patterns and Male Substrate Use

Activity patterns

We quantified the circadian activity cycle of female and male *S. retrorsa* in order to gain insight into the light levels that these spiders are most likely to experience during courtship. We monitored the locomotor activity of 16 mature female and 16 mature male *S. retrorsa* on an LD 12:12 h cycle for 5 days. This light cycle matched the cycle that they had experienced in the laboratory. Each spider was placed in a 25 mm diameter × 125 mm length glass tube, with half of a wet 10 × 38 mm dental cotton roll inside each end of the tube to provide moisture. Tubes were inserted into a locomotor activity monitor (model LAM) from Trikinetics, Inc. (Waltham, MA, U.S.A.), which houses four rows of eight tubes. Activity was recorded as the number of times spiders interrupted one of three infrared beams passing through the centre of each tube, counted in 5 min bins. Tubes were taped with masking tape to limit visual interactions between spiders, but a gap was left to permit the infrared beams to pass through the midline of each tube. The two ends of each tube were covered with a black vinyl cap. Lights went on at 0830 hours and off at 2030 hours. Light was provided with a full-spectrum compact fluorescent light bulb (NaturesSunlite 30 W, Naturallighting.com, Dickinson, TX, U.S.A.). Although there are no data available on the critical flicker fusion frequency of *S. retrorsa*, previous studies on another wolf spider, *Hogna baltimoriana*, demonstrated a critical flicker fusion frequency of 10 Hz, significantly lower than the flicker rate of a normal fluorescent bulb (120 Hz) (DeVoe, 1963; Inger, Bennie, Davies, & Gaston, 2014). To minimize disturbance and the influence of other light sources, the set-up was placed in an otherwise unused room accessible only through another dark room. The room was not entered throughout the duration of the experiment. We placed spiders in the activity monitor for 6 days and measured activity for days 2–6, thus allowing the spiders 1 day of acclimation.

Male substrate use

We compared male *S. retrorsa* habitat use across three substrate types (leaf litter, pine litter, sand) under two contexts: (1) general habitat use (no female cues present) and (2) courtship-specific habitat use (female cue present). Quantifying male substrate use under conditions that are likely to elicit courtship (i.e. female cue present) allowed us to test the hypothesis that males engage in microhabitat-specific courtship behaviour; a hypothesis not tested in previous work.

We constructed microhabitat choice arenas out of circular plastic containers (Pioneer Plastics, Inc., Dixon, KY, U.S.A.; 190 mm diameter, 70 mm height) equally divided into three sections. In each section, we placed one of the three substrate types (leaf litter, pine litter or sand; all collected at spider collection sites) at a depth of 20 mm. Spiders could readily cross between substrates. A circular central platform (30 mm diameter) connected all three substrate types. At the bottom of each substrate, we placed a contact microphone (35 mm diameter, Goedrum Co., Chanhua, Taiwan) connected to a Tascam DR-05 audio recorder (44.1 kHz sampling rate, TEAC, Wiesbaden, Germany). Contact microphones recorded vibratory signals across substrates (Appendix, Fig. A1).

We used a repeated measures design in which males were first run in the absence of a female cue and then 24 h later, run again in the presence of a female cue. This order was necessary to eliminate the possibility that female cues elicit long-term changes in mate searching. Although an alternative design could have run half of the males in the presence and half in the absence of female cues, we did not choose this design due to concerns over sample size.

Female cues consisted of female silk deposited on filter paper at the central platform. This was the only place that female silk was present (in cue present trials). The intention was for males to detect the female pheromone and begin courtship behaviour and mate searching (Tietjen, 1979). Female cues were obtained by placing mature virgin females on filter paper for 1 h prior to the experiment to accumulate silk on the paper. After 1 h, we cut the filter paper into circles (30 mm diameter) to place on the central platform. The presence of female cues on the central platform alone removed any potential confounding effects of female silk deposition across different substrates. We used 89 mature *S. retrorsa* males in both cue present and cue absent trials and all individuals were used 18 days after their maturation date.

To begin microhabitat use trials, males were placed within a transparent barrier of acetate film on the central platform. Once the spider remained motionless for a few seconds (mean ± SD time to motionless = 177.747 ± 123.540 s), we lifted the barrier and recorded movement and courtship for 30 min. Courtship was recorded by the contact microphones (see above) and a Sony HDR-SR10 video camera (Sony Electronics Inc., Minato, Tokyo, Japan) under two Vita-Lite full-spectrum 30 W fluorescent bulbs (Duro-Test Lighting Inc., Tultitlán, Mexico). Using a combination of video and acoustic recordings, we quantified (1) the initial substrate choice, (2) the number of substrate changes, (3) the residing time and (4) male courtship vigour – estimated by dividing the courting time by

the residing time on each substrate type. Courting time was measured as the sum of courting bouts defined from the beginning of pedipalpal drumming to the end of leg tapping (Hebets et al., 1996). Raven Pro v.1.5 for Windows (Cornell Lab of Ornithology, Ithaca, NY, U.S.A.) was used for analysing vibratory signal recordings. Between each trial, we replaced the substrate in each arena and cleaned the cardboard dividers, central platform and wall of the arena with distilled water. To monitor male body condition, we measured male body mass every day before experiments begin (Rundus et al., 2010).

Statistical analysis

We first performed McNemar's tests to see whether the initial choice of substrate was significantly different between female cue present and female cue absent treatments. We next performed generalized linear mixed effects model analyses on the relationship between female cues (presence/absence) and the male searching and courtship behaviour – estimated by the number of substrate changes, the presence of male courtship, the total courting time and the proportion of residing time on each substrate. As fixed effects, we included the presence of female cue in the model with individual as a random effect. Due to overdispersion, we modelled a negative binomial regression for the number of substrate changes and a gamma regression for the total courting time. We used beta logistic regression model for the proportion of residing time on each substrate. Also, to examine the effect of substrate type on male courtship vigour, we conducted a mixed effect beta regression analysis with individual as a random effect.

P values of fixed effects were estimated by likelihood ratio tests on the full model with the effect in question against the model without the effect. All the generalized linear mixed regression analyses were conducted using 'glmmTMB' functions from R package 'glmmTMB' (Brooks et al., 2017). For significant fixed effects, we subsequently conducted post hoc analyses using pairwise comparisons of estimated marginal means (hereafter, EMM) and log odds ratios for logistic regression using 'emmeans' functions from R package 'emmeans' (Lenth, 2018). *P* values were Bonferroni corrected.

Experiment 2: Substrate Type and Vibratory Signal Transmission

We added to our knowledge of substrate-dependent vibratory signal transmission in *S. retrorsa* by including an additional natural substrate type – sand. Notably, we also analysed vibratory signal components separately to assess component-specific signal transmission. Ultimately, we quantified the transmission of (1) pedipalpal drumming and (2) foreleg taps across three substrate types – leaf litter, pine litter and sand.

We quantified vibratory signal transmission across substrate types by playing recorded *S. retrorsa* vibratory displays through three different substrate types (oak leaf litter, pine litter and sand). We measured signal intensity at eight distances from the source (1, 5, 10, 20, 40, 80, 160 and 240 mm), using techniques adapted from Hebets et al. (2008). Measurements of signal intensity for different signal types (see below) were measured at each of the eight distances with a Polytec PSV–400 scanning laser vibrometer (Polytec Inc., Irvine, CA, U.S.A.).

We generated playback signals using recordings of live male *S. retrorsa* recorded on a stretched nylon substrate impregnated with female silk. Exemplar recordings were made at a distance of 1–2 mm from the male using a Polytec PSV–400 scanning laser vibrometer.

Male courtship consists of two distinct vibratory components: pedipalpal drumming and foreleg taps (Hebets et al., 1996; Hebets et al., 2013). These two components are acoustically distinct, and we therefore performed playbacks for them separately. We measured the transmission of drumming and tapping signals from three exemplar males across three replicates of each substrate type. For each replicate, we removed the substrate and reintroduced it, thus altering its arrangement. By including variation in substrate arrangement, we attempted to account for natural variability, which may significantly affect signal transmission. We played back signals through a 10 mm Samsung DMJBRN 1030BK linear resonant vibration actuator. Output from the actuator was calibrated using a digital equalization filter (Cocroft, Gogala, Hill, & Wessel, 2014). For leaf and pine playbacks, the actuator was affixed to the substrate with low-temperature hot glue. For the sand treatment, the actuator was pressed gently into the substrate. For all recordings, the actuator was positioned so that it was at the surface of the substrate.

We measured signal attenuation as the root mean square (RMS) amplitude of the signal (in dB) at different distances relative to the signal amplitude of the single loudest recorded measurement for each display component for a given male. This reference measurement was always at a distance of 1 mm from the actuator, usually on leaf litter. By comparing each point to the loudest recorded measurement, we thus compared signal attenuation at any given point to the maximum intensity for that signal. As such, these amplitudes are standardized across substrates, thus facilitating cross-substrate comparisons.

We measured the frequency filtering properties for each substrate by playing a sine sweep through the same actuator set-up described above and recording vibrations at three distances from the playback source (20 mm, 40 mm, 80 mm). The sine sweep excites all frequencies equally, and any differences in amplitude across the measured frequency range are therefore a result of filtering by the substrate. For each substrate, we measured intensity across the frequency range (in dB) for the three distances relative to the peak frequency at that distance. These measurements therefore represent the relative attenuation of all frequencies to the peak frequency on a given substrate and distance. We generated figures of these data using cubic smoothing spline fits. For comparison of these transfer functions, we also generated smoothing spline-fitted figures of the raw velocities measured across the frequency range for pedipalpal drumming and foreleg taps. Unlike the frequency filtering data, the magnitude of these velocities can be compared across distances and substrates.

Statistical analyses

We conducted a linear mixed effects analysis to test the influence of substrate on the attenuation of vibratory components produced by pedipalpal drumming, foreleg tapping and the full courtship display with individual replicate as a random factor. *P* values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. For modelling, we used 'lmer' functions from the R package 'lme4' (Bates, Maechler, Bolker, & Walker, 2015).

Experiment 3: Substrate Type, Light Level and Copulation Success

We compared substrate-specific courtship and copulation patterns among three substrate types (leaf litter, pine litter and sand) under two separate light conditions (light present and light absent). If prior results showing higher copulation success on pine litter and sand was due to increased visual signal transmission on these substrates, then we would expect this pattern to disappear in the absence of light.

Mating arenas consisted of circular plastic containers (diameter: 203 mm, height: 76 mm) filled to approximately 30 mm deep with one of the three substrate types. Between each trial, we completely

removed the substrate in each arena, rinsed the arena with distilled water and refilled them with new substrate. Light present trials were illuminated using two Vita-Lite full-spectrum 30 W fluorescent bulbs (Duro-Test Lighting Inc., Tultitlán, Mexico). Light absent trials were conducted in complete darkness.

The experiments involved 78 pairs – 13 pairs for each of six treatment combinations in 2×3 design: light present/absent on leaf litter, light present/absent on pine litter and light present/absent on sand. We used each spider only once. During each trial, a single female–male *S. retrorsa* pair was placed on each substrate type. Before males were placed in the experimental arena, females had 5 min of acclimation time. After males were placed in the arena, we observed behaviour for 45 min. We recorded courtship presence/absence, time to first courtship (i.e. latency), the number of male attempts to mount, copulation presence/absence and time to copulation. We considered the time from chemoexploration to the first pedipalpal drumming as the latency to first courtship and the time from chemoexploration to the first pedipalpal insertion as latency to copulation. We observed light absent trials with Rigel 3200 night vision goggles (Rigel Optics Inc., Washougal, WA, U.S.A.) or with a Sony HDR-SR10 night-shot camera (Sony Electronics Inc., Minato, Tokyo, Japan).

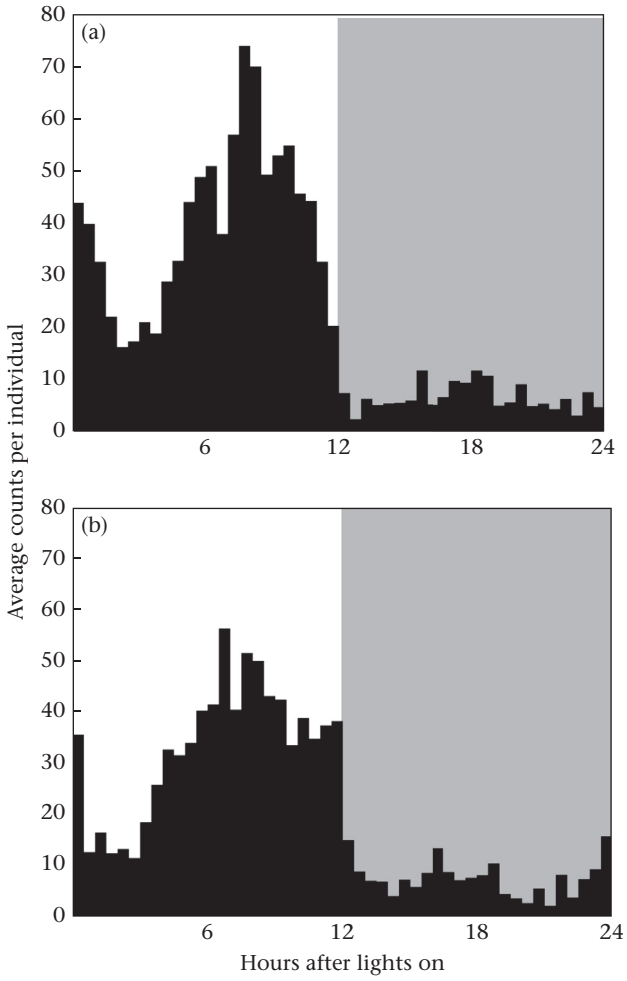

Figure 1. Entrainment profile for locomotor activity of (a) male ($N = 16$) and (b) female *S. retrorsa* ($N = 16$). Counts represent the average number of interruptions of an infrared beam per individual, in 30 min intervals, over 5 days of a 12:12 h light:dark cycle. Shaded background represents the dark period of the light:dark cycle.

Statistical analyses

We first used a binomial logistic regression model for the presence of male courtship/copulation and a Poisson regression model for the number of attempts by the male to mount the female to determine whether the behaviours were dependent on substrate type in different light conditions. Next, to accommodate all of our data, we used beta regression analyses to investigate the effect of substrate type, light condition and the interaction term on the proportional latency to male courtship behaviour and copulation. The proportional latency was calculated by dividing the starting time of courtship/copulation by the total duration of a trial. *P* values of each fixed effect were estimated by likelihood ratio tests of the full model with the effect in question against the model without the effect. All generalized linear mixed regression analyses were conducted using 'glmmTMB' functions from R package 'glmmTMB' (Brooks et al., 2017). For significant fixed effects, we subsequently conducted post hoc analyses using pairwise comparisons of estimated marginal means (hereafter, EMM) and log odds ratios for logistic regression using 'emmeans' functions from R package 'emmeans' (Lenth, Singmann, Love, Buerkner, & Herve, 2019). *P* values were Bonferroni corrected.

Ethical Note

No special protocols are required for the handling of use of spiders in research. We nevertheless ensured the proper maintenance and ethical care for all spiders in our study. Collecting permits (0706161) were provided by the Mississippi Department of Wildlife Fisheries and Parks. After experiments, spiders were individually euthanized by freezing, and then preserved in 70% ethanol. To the best of our knowledge, we followed the suggestions of ASAB/ABS Guidelines for the use of animals in research.

RESULTS

Experiment 1: Activity Patterns and Male Substrate Use

Activity patterns

Both females and males showed peak activity in the light (Fig. 1). Males were most active 7–8 h after lights on, and female activity was the highest 1 h earlier than that (6–7 h after lights on).

Male substrate use

There was no change in body mass of spiders during the experimental period (Wilcoxon signed-rank test: $W = 1702.5$, $Z = 0.195$, $P = 0.847$). The presence of female cues did not affect the initially chosen substrate type (McNemar's test: leaf litter: $P = 0.311$; pine litter: $P = 0.728$; sand: $P = 0.174$).

The presence of female cues had significant effects on the number of substrate changes ($\chi^2_1 = 17.633$, $P < 0.001$), the presence of male courtship ($\chi^2_1 = 18.242$, $P < 0.001$) and the total courting time ($\chi^2_1 = 10.980$, $P = 0.001$). In the presence of female cues, changes among substrates were more frequent (EMM ± SE: presence: 11.93 ± 1.477; absence: 6.65 ± 0.866; $t = -4.46$, < 0.001), more males courted (EMM ± SE: presence: 0.245 ± 0.096; absence: 0.035 ± 0.034; $t = -3.041$, $P = 0.003$) and courtship lasted longer (EMM ± SE: presence: 205.1 ± 36.7; absence: 42.9 ± 14.4; $t = -4.121$, $P < 0.001$).

Male courtship vigour was influenced by substrate type ($\chi^2_2 = 35.601$, $P < 0.001$) and presence of the female cue ($\chi^2_2 = 14.742$, $P = 0.002$). Males courted less vigorously on sand (EMM ± SE = 0.015 ± 0.005) than on leaf litter (EMM ± SE = 0.040 ± 0.014) and pine litter (EMM ± SE = 0.063 ± 0.020) (versus leaf litter: $t = 3.958$, $P < 0.001$; versus pine litter: $t = 5.149$, $P < 0.001$). There was no significant difference in male courtship vigour

between leaf litter and pine litter ($t = -1.233$, $P = 0.662$). Also, across substrate types, male courtship was more vigorous in the presence of the female cue (EMM ± SE = 0.064 ± 0.015) than in the absence of the female cue (EMM ± SE = 0.017 ± 0.008; $t = -3.589$, $P = 0.001$).

The presence of female cues had a significant influence on the proportion of residing time on sand ($\chi^2_1 = 4.759$, $P = 0.029$) but not on leaf litter ($\chi^2_1 = 0.181$, $P = 0.671$) or pine litter ($\chi^2_1 = 2.304$, $P = 0.129$). The proportion of residing time on sand was significantly larger in the presence of the female cue (EMM ± SE = 0.118 ± 0.022) than in the absence of the female cue (EMM ± SE = 0.082 ± 0.016) ($t = -2.191$, $P = 0.030$; Fig. 2). Courting time on each substrate was significantly correlated with residing time across all the substrates (Spearman's correlation: leaf: $r_S = 0.576$, $P = 0.001$; pine: $r_S = 0.743$, $P < 0.001$; sand: $r_S = 0.464$, $P = 0.011$).

In summary, the presence of female cues elicited male mate searching and courtship behaviour and influenced male substrate use. Although males resided mostly on leaf litter, males tended to invest more time on sand in the presence of female cues.

Experiment 2: Substrate Type and Vibratory Signal Transmission

There was a significant effect of substrate type on signal attenuation by distance. Using both signal components in the model, attenuation was dependent on substrate type and on the interaction term between substrate type and distance (substrate: $\chi^2_4 = 398.96$, $P < 0.001$; substrate∗distance: $\chi^2_2 = 10.975$, $P = 0.004$). Using only pedipalpal drumming in the model, attenuation was also dependent on substrate type and the interaction term between substrate and distance (substrate: $\chi^2_4 = 200.060$, $P < 0.001$; substrate∗distance: $\chi^2_2 = 6.384$, $P = 0.041$). The effect of the interaction term was not significant in the model with only leg taps (substrate: $\chi^2_4 = 202.160$, $P < 0.001$; substrate∗distance: $\chi^2_2 = 4.898$, $P = 0.086$). Vibratory signals were transmitted best on leaf litter, followed by pine litter and then by sand, regardless of structural variation (all components: leaf litter versus pine litter: $t = 7.322$, $P < 0.001$; leaf litter versus sand: $t = 14.876$, $P < 0.001$; pine litter versus sand: $t = 17.265$, $P < 0.001$; pedipalpal drumming: leaf litter versus pine litter: $t = 5.021$, $P < 0.001$; leaf litter versus sand: $t = 17.319$, $P < 0.001$; pine litter versus sand: $t = 12.298$, $P < 0.001$; leg tap: leaf litter versus pine litter: $t = 5.332$, $P < 0.001$; leaf litter versus sand: $t = 17.469$, $P < 0.001$; pine litter versus sand: $t = 12.137$, $P < 0.001$) (Fig. 3).

Comparing frequency filtering across substrate types (Fig. 4a–c), leaf and pine litter acted as low-pass filters, significantly attenuating frequencies above 1000 Hz. Conversely, sand did not appear to filter any specific frequencies. The lack of frequency filtering on sand most likely occurred because sand does not pass any frequencies well. At 20 mm from the source on sand, the amplitude of both taps and drumming were less than at 80 mm from the source on both leaf and pine. Comparing the velocities of drumming and tapping playbacks across the three substrate types (Fig. 4d–i), we found that *S. retrorsa's* courtship mostly resided in the 1–500 Hz range, which transmits well through both leaf and pine. We also found evidence that the peak frequencies of both drumming and tapping shifted towards lower frequencies at greater distances from the source, potentially as a result of the low-pass nature of the substrate.

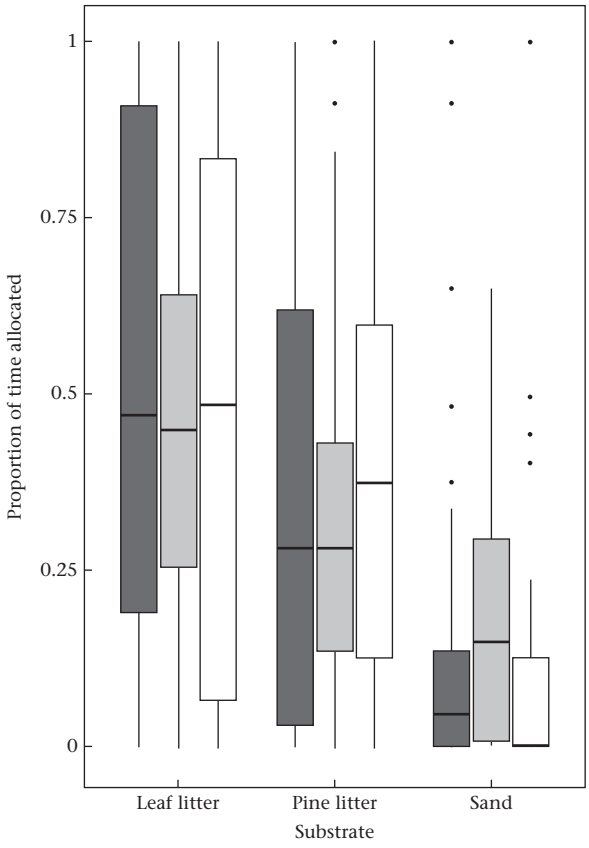

Figure 2. Residing and courting time allocation among substrates ($N = 65$). Black bars: proportion of residing time in the absence of the female cue; grey bars: proportion of residing time in the presence of the female cue; white bars: proportion of courting time among courting individuals.

Experiment 3: Substrate Type, Light Level and Copulation Success

The likelihood of males courting was not influenced by substrate type ($\chi^2_4 = 4.615$, $P = 0.329$), light condition ($\chi^2_3 = 3.236$, $P = 0.357$) or any interactions ($\chi^2_2 = 3.016$, $P = 0.221$; Fig. 5a). The likelihood of copulation was significantly influenced by substrate type ($\chi^2_4 = 10.127$, $P = 0.038$) but not by light condition ($\chi^2_3 = 2.128$, $P = 0.546$) or any interactions ($\chi^2_2 = 2.071$, $P = 0.355$). Spiders were more likely to copulate on sand (EMM ± SE = 0.748 ± 0.090) than on leaf litter (EMM ± SE = 0.345 ± 0.094; $t = -2.730$, $P = 0.024$). The likelihood of copulation on pine litter (EMM ± SE = 0.500 ± 0.098) was not significantly different than that on other substrates (versus leaf litter: $t = -1.121$, $P = 0.798$; versus sand: $t = -1.755$, $P = 0.250$; Fig. 5c).

The number of males attempting to mount females was not dependent on substrate type ($\chi^2_4 = 2.375$, $P = 0.667$), light condition ($\chi^2_3 = 2.101$, $P = 0.552$) or interactions ($\chi^2_2 = 0.816$, $P = 0.665$).

The latency to first courtship was significantly influenced by substrate type ($\chi^2_4 = 9.537$, $P = 0.049$) but not by light condition ($\chi^2_3 = 7.456$, $P = 0.059$) or interactions ($\chi^2_2 = 5.335$, $P = 0.069$). However, a post hoc pairwise comparison did not show a significant difference between substrates (leaf litter versus pine litter: $t = -0.716$, $P = 1.000$; leaf litter versus sand: $t = -2.187$, $P = 0.097$; pine litter versus sand: $t = -1.439$, $P = 0.465$; Fig. 5b). The latency to copulation did not differ between the two light conditions ($\chi^2_3 = 2.376$, $P = 0.498$), but there was a significant difference in the latency to copulation between substrate types ($\chi^2_4 = 10.056$, $P = 0.040$). There was no significant effect of the interaction term on the latency to copulation ($\chi^2_2 = 2.145$, $P = 0.342$). The latency to copulation on sand (EMM ± SE = 0.778 ± 0.049) was significantly shorter than on leaf litter (EMM ± SE = 0.901 ± 0.254; $t = 2.928$, $P = 0.014$). There was no significant difference in the latency to copulation between pine litter (EMM ± SE = 0.877 ± 0.031) and

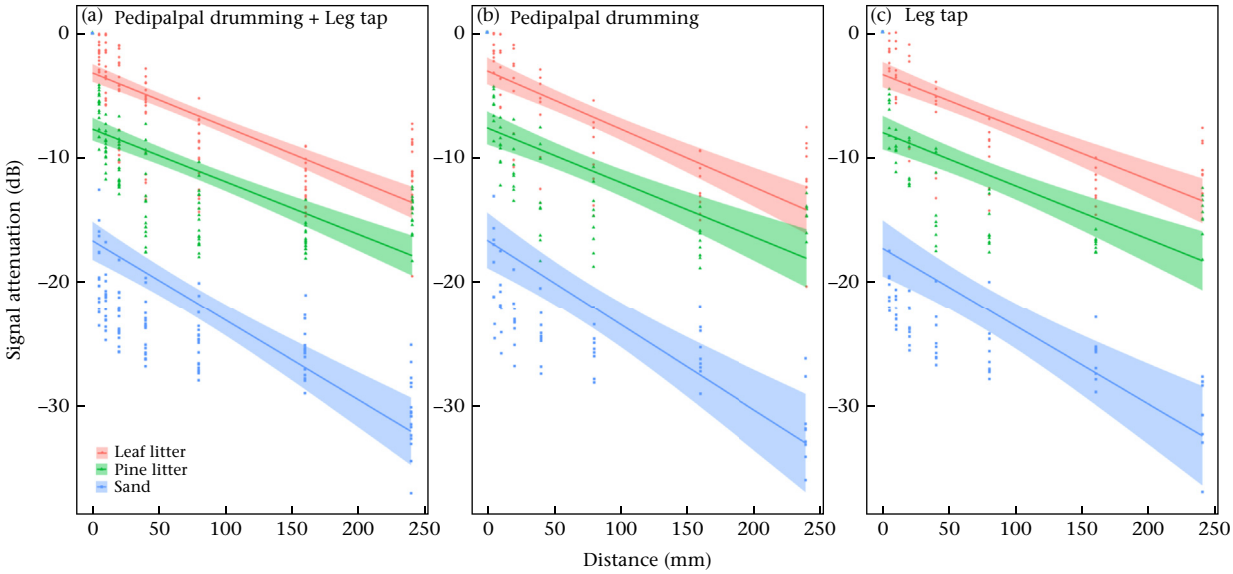

Figure 3. Signal attenuation by distance among substrates. Bands denotes 95% confidence intervals.

other substrates (versus leaf litter: $t = 0.772$, $P = 1.000$; versus sand: $t = 2.176$, $P = 0.099$; Fig. 5d).

DISCUSSION

The signalling environment that *S. retrorsa* males experience, in terms of both time (of day) and space (substrate type), influences their activity patterns, their vibratory signal transmission, their visual signal transmission and their copulation success. The influence of the signalling environment, however, does not conform to predictions of the sensory drive hypothesis (i.e. we found no signal–substrate match). We found that female and male *S. retrorsa* appear to be most active during daylight hours, and mature males reside longer on leaf litter, as compared to pine litter or sand regardless of the presence/absence of female cues. Additionally, vibratory courtship signals transmitted best, in terms of attenuation, on leaf litter. While these results would predict that leaf litter would yield the highest copulation success, instead, we found that males obtained the highest copulation success on sand, regardless of the light level. Consistent with prior research on *S. retrorsa*

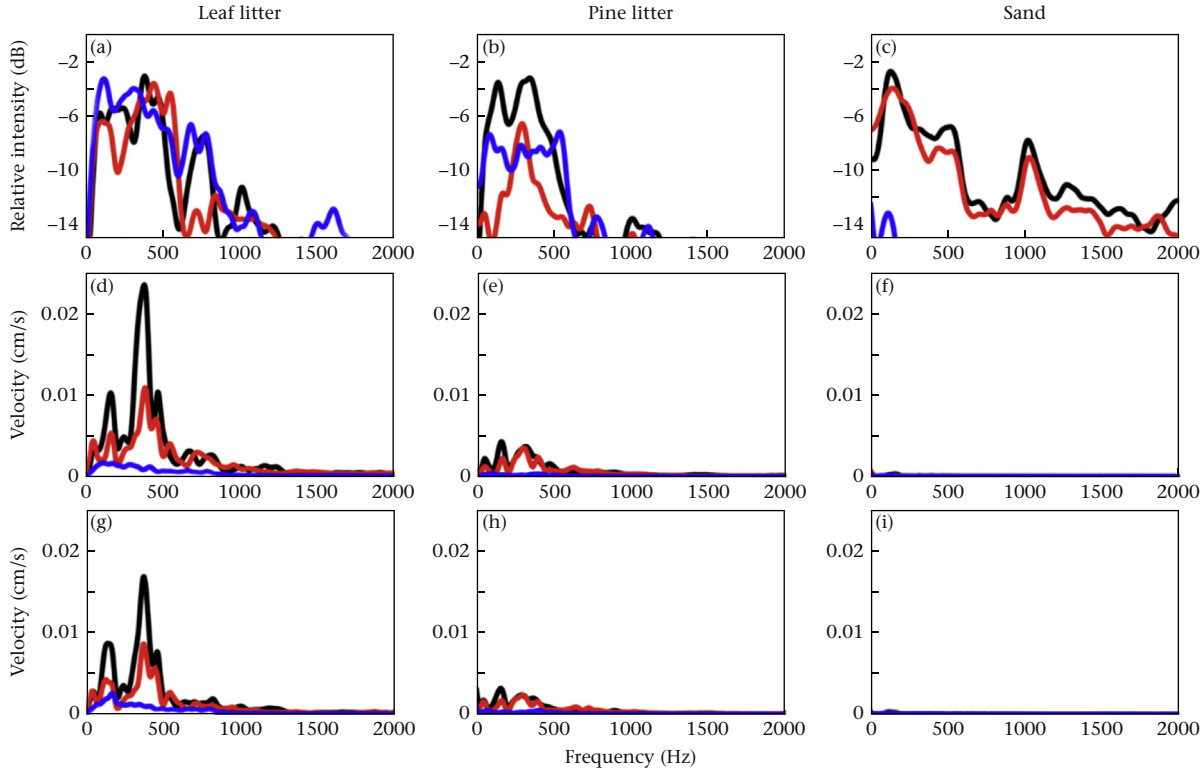

Figure 4. The frequency filtering properties of (a) oak leaf litter, (b) pine litter and (c) sand, calculated from sine sweep playbacks recorded at 20 mm (black), 40 mm (red) and 80 mm (blue) from the source. Measurements were normalized as the relative decibel loss compared to the peak frequency within a given substrate and distance. Measurements of velocity for (d–f) drumming and (g–i) tapping on the same substrates and at the same distances are also shown.

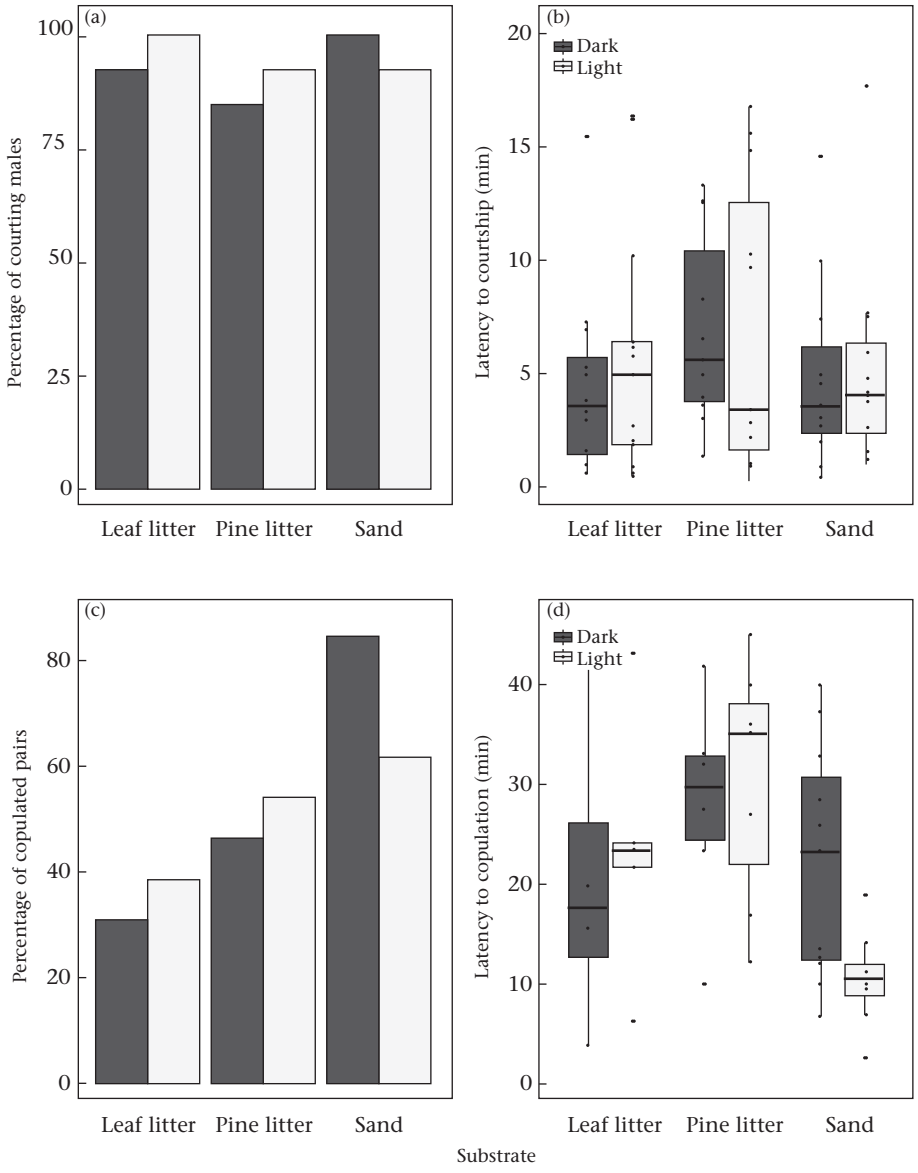

Figure 5. Male courtship behaviour and copulation across substrates between light and dark environments ($N = 13$ for each category): (a) the percentage of courting males, (b) the latency to courtship signalling, (c) the percentage of copulated pairs and (d) the latency to copulation. Dark grey bars: individuals in the dark environment; light grey bars: individuals in the light environment.

(Hebets et al., 2008; Hebets et al., 2013; Rundus et al., 2010), but in contrast to data on other *Schizocosa* species (Elias et al., 2010; Gordon & Uetz, 2012; Hebets, 2008; Hebets et al., 2013; Rosenthal et al., 2019), our results support the hypothesis that neither vibratory nor visual courtship signals are necessary for *S. retrorsa* copulation success. Additionally, while the microhabitat (i.e. substrate type) influences copulation success in *S. retrorsa*, this influence is not through its effects on visual or vibratory signal transmission. Together, our results are inconsistent with the predictions of sensory drive.

Activity Patterns and Microhabitat Use

Our activity data suggest that *S. retrorsa* females and males are most active in daylight hours – implying the potential for visual signalling to play an important role in courtship. In terms of activity across microhabitats, males resided longest on leaf litter and courted more vigorously on leaf and pine litter than on sand. Not surprisingly, courting time was correlated with residing time. These results are similar to a previous study that used subadult and adult female and male *S. retrorsa*. The prior study found that the first substrate choice and residing time did not differ between substrate types for any of the groups, but spiders tended to reside longer on leaf litter (Hebets et al., 2008). These authors also observed that adult males moved more than subadults and they suggested that this may be driven by mate searching (Hebets et al., 2008). In support of this hypothesis that the increased movement is due to mate searching, when a female cue was present (versus absent), mature males changed substrates more times. Field research on the locomotion of wolf spiders also supports the hypothesis that mature males move more than females (*S. ocreata*; Cady, 1984; *Venatrix lapidosa*; Framenau, 2005).

Schizocosa retrorsa males generally resided longer on leaf litter and courted more vigorously, despite leaf litter being the substrate on which they were least likely to mate. We suspect that differences in residing time and courtship vigour across substrates reflect the

different physical structures of the microhabitat rather than courtship location preferences. By controlling the depth of substrates, we unintentionally created microhabitats with drastically different physical structure and surface area. Our leaf litter consisted of dried oak leaves and twigs, each with distinct three-dimensional properties. This environment was a complex, three-dimensional labyrinth of spaces that males (and females had they been present) could hide in and/or explore. Pine needles had relatively less structural variation than deciduous leaf litter, but nevertheless, there was still three-dimensional spaces created. In stark contrast, spiders in the sand were restricted to a two-dimensional horizontal plane of movement. Ultimately, leaf litter most likely had the largest possible surface area for males to manoeuvre, followed by pine litter, then sand. This matches the residing time observed by males in this study. Taken together with the context-dependent substrate choice (i.e. males resided longer on sand when they were exposed to female cues), it is possible that our observed residing time does not reflect microhabitat preferences, but instead reflects movement patterns related to structural complexity.

Note, however, that our experimental design could not control for the potential impact of a time effect between female cue presence/absence trials. Experience with the experimental set-up may have influenced a male's subsequent behaviour – e.g. making him more acclimated and thus more likely to move or court the second time. Nevertheless, in both sets of trials, there was no significant difference in the pattern of male substrate use, suggesting that males behaved similarly across time.

Vibratory Signal Transmission Across Substrate Types

Unsurprisingly, leaf litter, pine litter and sand had different vibratory transmission properties. Our sine sweep experiment, which allowed us to generate transfer functions for each substrate, demonstrated that the three substrate types filter frequencies differently. In particular, leaf litter and pine litter were low-pass filters. In relation to leaf litter and pine litter, sand did not appear to differentially filter frequencies but instead attenuated all vibratory signals to a higher degree than pine or leaf litter habitats. Notably, on sand, energy in vibratory signals was indistinguishable from the noise floor at 20 mm. When we compared the transmission of a spider's actual song across substrate types – both drumming and leg tapping – we similarly found that the substrates attenuated drumming and leg tapping to different degrees. In particular, the attenuation of drumming was dependent on substrate type, whereas foreleg tapping was independent of substrate. This suggests that foreleg tapping might be a more effective signal across variable environmental conditions whereas pedipalpal drumming is more effective in particular signalling locations. Also, we found evidence that frequency filtering alters the frequency spectra of *S. retrorsa* drumming and leg tapping on leaf litter. Together, our data suggest that leaf litter and pine litter are good low-pass filters, that leaf litter alters the vibratory song and that sand is similar to a vibration absent signalling environment (Fig. 4).

Copulation Success Across Substrate Types and Light Levels

When confined to distinct substrate types, *S. retrorsa* pairs were more likely to mate on sand than on leaf litter, while copulation success on pine litter did not differ from that on the other substrates tested. This same pattern of mating was seen regardless of the light conditions. This substrate-dependent copulation pattern is similar to that found in a previous study that used leaf litter, pine litter and red clay as substrate types (Hebets et al., 2008). Our results suggest that any variation in the transmission efficacy of vibratory or visual signalling (which we did not quantify here) across substrate types cannot explain substrate-dependent copulation success. These results are also consistent with previous work suggesting that the light environment had no influence on copulation success of *S. retrorsa* (Hebets et al., 2013; Rundus et al., 2010).

We found a mismatch between the best substrate type for vibratory signal transmission and the substrate upon which *S. retrorsa* obtained the highest copulation success. This mismatch was not limited to effective vibratory signalling and copulation success, but included visual signalling as well. Approximately 80% of pairs mated in complete darkness on sand (Fig. 3) – i.e. in the effective absence of both vibratory and visual signal perception. The copulation success of *S. retrorsa* on sand in the absence of visual signals was much higher than that reported for *S. retrorsa* on granite in the same light environment (27% in Rundus et al., 2010, 26% in Hebets et al., 2013). Although structural complexity might help explain low levels of copulation success on leaf and pine litter (see earlier discussion of microhabitat preference), pairs remain capable of mating in the absence of vibratory and visual signals. Similar mismatches have been observed in other communication systems as well (Jain & Balakrishnan, 2012; Schmidt & Balakrishnan, 2015; Smith et al., 2011; Smith, van Staaden, & Carleton, 2012; Zhao et al., 2017).

Summary

In stark contrast to other *Schizocosa* species (Elias et al., 2010; Hebets et al., 2013), microhabitat use and vibratory and visual transmission properties cannot explain substrate-dependent mating success in *S. retrorsa.* Assuming that our experimental design is sufficient, we propose the following possibilities. (1) Male *S. retrorsa* 'courtship displays' do not influence copulation success (2) alternative signalling modalities (e.g. air particle displacement or chemical signalling), play a role (3) other substrate-dependent factors (e.g. perceived predation risk), influence female preference for male vibratory signalling and copulation success and/or (4) females exhibit a reversed preference (i.e. deterrence) for vibratory signalling.

The first potential explanation for the mismatch between signal transmission and signal function (i.e. successful copulation) is that the conspicuous (to human observers) vibratory and visual courtship signalling in *S. retrorsa* no longer function in courtship. If the courtship display serves only a minor role in *S. retrorsa* copulation, for example, the substrate-dependent copulation success may be an outcome of the difference in the chance that males randomly run into females in different physical structures of microhabitats – i.e. more likely on sand. We find this explanation highly unlikely though, as male courtship is initiated by contact with female silk cues alone (Rundus, Biemuller, DeLong, Fitzgerald, & Nyandwi, 2015), and male courtship vigour is the best predictor of mating success in *S. retrorsa* (Rundus et al., 2010). Furthermore, the number of attempted mounts did not significantly differ between signalling environments, suggesting that the substrate-dependent copulation success was caused by female responses to male signal, not by other factors such as encounter rate in structurally different substrates.

Second, our study focused on vibratory and visual signalling, but spiders may also rely on other sensory modalities during courtship – e.g. air particle movement or airborne chemical cues. For instance, female silk contains chemical cues of the female's reproductive state, receptivity or age (Roberts & Uetz, 2005; Rundus et al., 2015). While females may alter their silk cues across substrate types, this possibility would require future testing. The possibility that *S. retrorsa* use air particle movement (near-field sound), however, has already received some attention.

Previous studies have suggested that air particle movement is probably used by many insects and arthropods (Barth, Humphrey, Wastl, Halbritter, & Brittinger, 1995; Cator, Ng'Habi, Hoy, & Harrington, 2010; Klopsch, Kuhlmann, & Barth, 2013; Lapshin & Vorontsov, 2017; Santer & Hebets, 2008; Santer & Hebets, 2011; Shimozawa & Kanou, 1984). Some argue that the ability of animals to use air particle movements to guide behaviour has been dramatically underestimated (Barth & Höller, 1999; Bathellier, Steinmann, Barth, & Casas, 2012; Klopsch et al., 2013; Raboin & Elias, 2019; Shamble et al., 2016). In line with this suggestion, Rundus et al. (2010) previously suggested that the air particle movement generated by the leg waving during *S. retrorsa* courtship may play a role in courtship signalling. Their hypothesized role of air particle displacement was based upon prior findings that documented (1) the maintenance of male copulation success in the absence of visual and vibratory courtship components (similar to results of the current study) and (2) a positive relationship between the number of leg waves and copulation success (not tested in the current study). The authors further demonstrated mathematically that the leg waving of *S. retrorsa* could generate air particle movement that was of sufficient velocity to be detected by sensory hairs on the female from a distance of 65 mm (Rundus et al., 2010). While we did not quantify the potential for air particle displacement across our substrate types in the present study, we expect that it would have high efficacy on red clay and sand and lower transmission efficacy on pine litter and leaf litter – a pattern that matches the copulation success in the current study as well as prior research (Hebets et al., 2008). Similar to Rundus et al. (2010), we suspect a significant role of air particle displacement in *S. retrorsa* courtship – a hypothesis that is not mutually exclusive of others.

Third, other substrate-dependent factors may have been responsible for our observed substrate-dependent mating success. In *Schizocosa* wolf spiders, for example, predation risk during courtship signalling and copulation is most likely high due to decreased vigilance (Hebets, 2005), eavesdropping by predators (Roberts, Taylor, & Uetz, 2007) or prolonged copulation (1–8 h; Stratton, Hebets, Miller, & Miller, 1996). Furthermore, in other wolf spiders, courtship is known to increase predation risk. Courting male *Rabidosa punctulata* wolf spiders, for example, are attacked by heterospecifics more often than noncourting males (Wilgers, Wickwire, & Hebets, 2014). Due to this risk, males may choose to decrease the active space of their signals (Long & Rosenqvist, 1998) or alter their courtship behaviour. As predicted, *R. punctulata* males switch from a courtship mating tactic to a direct mount tactic in the presence of predatory heterospecific cues (Wilgers et al., 2014). Along the same lines, under natural conditions where predators are present, *Schizocosa retrosa* females may actively avoid copulating on leaf litter, which may increase predation risk or interrupt mate assessment by the enhanced vibratory signal conspicuousness. Consistent with this hypothesis, in a recent review, Schmidt and Balakrishnan (2015) suggested that other ecological factors such as predation may drive animals to produce signals in suboptimal signalling microhabitats (in terms of signal transmission).

Fourth, female *S. retrorsa* may be overly sensitive to conspecific vibratory signals or actively dislike them. All of our results are consistent with the notion that females avoid males that they can hear well, regardless of the light environment. Given that *S. retrorsa* females predominantly live on substrates that may dampen vibratory cues (e.g. sand), they may be frightened, startled or repelled by loud vibrations such as those produced by males courting on leaf litter. Alternatively, or in addition, attending to vibratory signals may be costly for *S. retrorsa* females. It may somehow reduce their ability to 'choose' among males, or may increase their risk of predation due to eavesdropping predators. Such costs might have led to female resistance to vibratory signalling over evolutionary time in an antagonistic, chase-away fashion (Cordero & Eberhard, 2005; Holland & Rice, 1998). Unfortunately, we are unable to assess these possibilities given our present data.

Finally, in addition to the previously discussed hypotheses, we can also include possibilities of reproductive character displacement (upland chorus frog, *Pseudacris feriarum*: Malone, Ribaldo, & Lemmon, 2014) and physiological plasticity (Malawi cichlids: Smith et al., 2012). At this time, however, the use of an additional sensory modality – air particle displacement – in *S. retrorsa* courtship is our favoured hypothesis to explain the seeming mismatch between vibratory and visual signal transmission and mating success.

In conclusion, like other studies that demonstrate equivocal support for sensory drive (Ey & Fischer, 2009; Malone, Ribado, & Lemmon, 2014; Schmidt & Balakrishnan, 2015; Smith et al., 2012), our results do not support a match between effective multimodal (vibratory and visual) signal transmission and signal function (copulation success) in *S. retrorsa*. We suggest that the discordance between the theoretical predictions and empirical tests of sensory drive may arise when factors other than mate choice take precedence, or when other (less conspicuous) modalities are at play. Nevertheless, our results, in combination with earlier studies, clearly demonstrate interspecific variation in the dominance and function of specific sensory modalities across *Schizocosa* multimodal courtship displays (Elias et al., 2010; Gordon & Uetz, 2011; Hebets, 2008; Hebets et al., 2013; Stratton, 2005; present study). Such interspecific variation suggests that different selective regimes have acted across species and that we need to broaden our hypotheses regarding complex signal evolution beyond that of sensory drive and mate choice, and beyond the traditional, conspicuous sensory modalities.

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Appendix

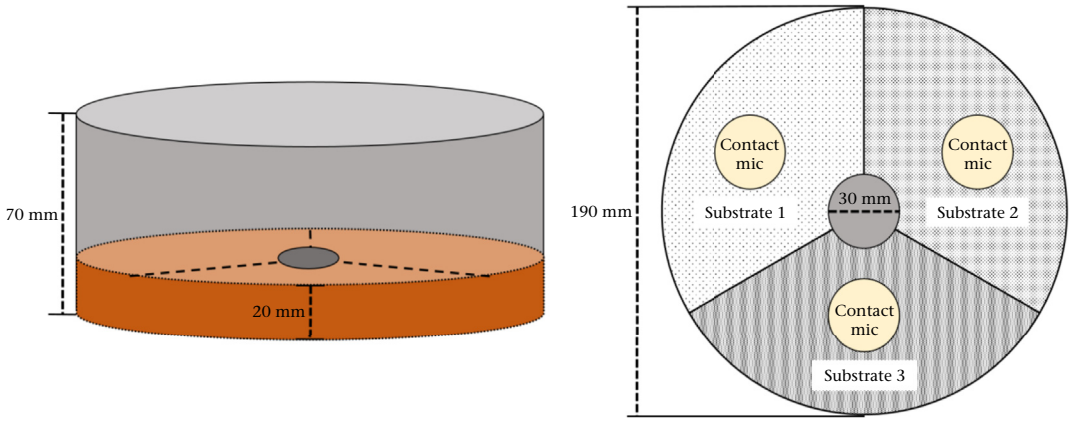

Figure A1. The design of an experimental arena for male substrate use experiment. A circular plastic container (190 mm diameter, 70 mm height) equally divided into three sections filled with one of the three substrate types (leaf litter, pine litter or sand) at a depth of 20 mm. A circular central platform (30 mm diameter) connected all three substrate types. A contact microphone (35 mm diameter) connected to an audio recorder was deployed at the bottom of each section.